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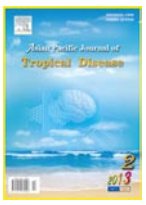

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Toxicity effect of *Delonix elata* (Yellow Gulmohr) and predatory efficiency of Copepod, *Mesocyclops aspericornis* for the control of dengue vector, *Aedes aegypti*

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PEER REVIEW

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Comments

This paper is significant for mosquito vector control especially insect not able to resistant. Synergistic study of plant species regulate the insect control mechanism is one of the significant study in this paper.
(Details on Page 125)

ABSTRACT

Objective: To evaluate the toxicity, predatory efficiency of *Delonix elata* (*D. elata*) and *Mesocyclops aspericornis* (*M. aspericornis*) against dengue vector, *Aedes aegypti* (*Ae. aegypti*). **Methods:** A mosquitocidal bioassay was conducted at different concentration of plant extract followed by WHO standard method. The probit analysis of each tested concentration and control were observed by using software SPSS 11 version package. The each tested concentration variable was assessed by DMRT method. The predatory efficiency of copepod was followed by Deo *et al*., 1988. The predator, *M. aspericornis* was observed for mortality, abnormalities, survival and swimming activity after 24 h treatment of plant and also predation on the mosquito larvae were observed. **Results:** *D. elata* were tested for biological activity against the larvae, and pupae of *Ae. aegypti*. Significant mortality effects were observed in each life stage. The percentage of mortality was 100% in first and second instars whereas 96%, 92% in third and fourth instars. Fitted probit-mortality curves for larvae indicated the median and 90% lethal concentrations of *D. elata* for instars 1-4 to be 4.91 (8.13), 5.16 (8.44), 5.95 (7.76) and 6.87 (11.23), respectively. The results indicate that leaf extract exhibits significant biological activity against life stages. The present study revealed that *D. elata* is potentially important in the control of *Ae. aegypti*. Similar studies were conducted for predatory efficiency of Copepod, *M. aspericornis* against mosquito vector *Ae. Aegypti*. This study reported that the predatory copepod fed on 39% and 25% in I and III instar larvae of mosquito and in combined treatment of *D. elata* and copepod maximum control of mosquito larval states and at 83%, 80%, 75% and 53% in I, II, III and IV instars, respectively. **Conclusions:** The combined action of plant extract and predatory copepod to effectively control mosquito population and reduce the dengue transmitting diseases.

KEYWORDS

Delonix elata, *Aedes aegypti*, *Mesocyclopes aspericornis*, Mortality, Predatory Efficiency

1. Introduction

Dengue is transmitted by mosquitoes of the genus *Aedes* which are widely distributed in subtropical and tropical areas of the world and is classified as a major global health threat by the World Health Organization[1]. Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *Aedes aegypti* (*Ae. aegypti*) and bite during the daytime. Mosquitoes are a serious threat to public health transmitting several dangerous diseases for over 2 billion people in the tropics[2]. *Ae. aegypti*, the primary carrier for viruses that cause dengue fever, dengue hemorrhagic fever and yellow fever are widespread over large areas of the tropics and subtropics and is reported to infect more than 100 million people every year in more than 110 countries in the tropics[3].

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Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe[4], in expensive and are readily available medicinal plants for mosquitoes control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. Larvicidal activities of the plant extracts varied based on the plant species, the plant parts, the geographical location where the plants grown and its application method. Neem products are capable of producing multiple effects in insects. Because of a variety of components affect different mechanism, insecticide resistant to this compound. Neem components affects different insects as well as medically important insects like mosquitoes, flies *etc*. Plant materials offer not only effective mosquito control agents, but also show environmental safety[5].

Similarly, the most effective species of adult copepod, *Mesocyclops aspericornis* (*M. aspericornis*) usually reduce larval survival by 99%-100%. Because the larvae are not merely thinned but substantially reduced, the production of adult mosquitoes is reduced correspondingly. One limitation of some copepod species is their tendency for unrestrained population growth in container habitats which lead to depletion of the food supply, stunting, and copepods that are too small to prey on mosquito larvae. The number of copepods in a container depends on the food supply. Most containers that have enough natural food to support mosquito production also have enough food to support a large copepod population. The *Delonix elata* (*D. elata*) plant had antimicrobial properties on bacteria and virus and not harmful to the copepod but suppress the survival of mosquito by means of respiration and brought out to sluggishness movement and finally cause death of mosquito larva.

Biological control of mosquito larvae with predators and other biocontrol agents would be a more effective and eco-friendly approach than the use of synthetic chemicals and reduce concomitant damage of insecticide applications to the environment[6]. Naturally, larvivorous and adult copepods were found everywhere in artificial containers, peridomestic containers, tyres and coconut shells *etc.*, which are the main breeding habitats of *Ae. aegypti*, but even both are present in same container the mosquito control was very less. In order to kill more number of larval instars of mosquito, an integrative effect of safer method is necessary to suppress the larval movement and copepods is very easier to kill more larvae and gain access to them. Hence, the present study investigated the potential effect of plant compound and predatory efficiency of copepod was good biological control agent against mosquito larvae of *Ae. aegypti*.

2. Materials and methods

2.1. Collection of plant materials

The plants was collected during the flowering season from Yercaud Hills, Salem District, Tamil Nadu, India and it was taxonomically identified at Botanical Survey of India, South Circle, TNAU Campus, Coimbatore and vouched. The identified plant, *D. elata* (L) Gamble preserved and deposited in laboratory at Department of Zoology, Bharathiar University, Coimbatore, India.

2.2. Preparation of plant extracts

The whole plant parts (leaves, flowers and twigs) were washed with tap water and shade dried at room temperature and with the help of an electrical mixer powdered the dried plant. From each sample, 200 g of the plant materials were extracted with 500 mL of solvents such as acetone and ethanol using the Soxhlet apparatus for 8 h[7].

2.3. Preparation of required concentration of plant extracts

One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) considered as 1% stock solution. Five different concentrations were prepared from the stock solution ranging from 2% to 10%.

2.4. Collection of mosquito eggs

Eggs of mosquito species were collected from National communicable disease centre, Mettupalayam, Coimbatore, Tamilnadu. These eggs were brought to the laboratory and transferred to 18 cm×13 cm×4 cm size enamel trays containing 500 mL of water and kept for larval hatching.

2.5. Maintenance of larvae

The mosquito culture was maintained in our laboratory at (27±2) °C, 75%-85% RH, Under 14L: 10 D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the larvae transformed into the pupae.

2.6. Maintenance of pupae and adult

The pupae collected from the culture trays were transferred to plastic containers containing 500 mL of water with the help of a dipper. The plastic jars were kept in 90 cm×90 cm ×90 cm size mosquito cage for adult emergence. The cage made up of wooden frames and covered with polythene sheets with four sides (two laterals, one back and other one upper) and the front part covered with a muslin cloth. The bottom of the cage was fitted with strong cardboard. The freshly emerged adults were maintained at (27±2) °C, 75%-85% RH, less than 14L: 10D photoperiod cycles. The adults were fed up with 10% sugar solution for a period of three days before providing animal blood for feeding.

2.7. Blood feeding of adult mosquito species

The adult female mosquitoes are allowed to feed blood of a rabbit (showed on the dorsal side) for 2 d, to ensure adequate

blood feeding for 5 d. After blood feeding enamel trays with water from the culture trays will be placed in the cage for the adults to lay eggs.

2.8. Collection of copepod

Copepod was collected from the pond in Muthannankulam, Coimbatore during early morning before sun rise. To collect the copepod from the pond, standard plankton mesh net with 100 μm was used. Collected copepod in 200 mL of plastic bottle were detached and transferred to the laboratory.

2.9. Identification of copepod in laboratory

Collected copepods from the pond were transferred into laboratory and cultured following[8]. The predatory nature and the rate of predation of *M. aspericornis* on mosquito larva were observed under a stereomicroscope. The morphological and taxonomic characters of copepod were identified using 10× Triocular stereo microscopes. Copepods in the laboratory were identified based on distribution of feathered and non-feathered outgrowths on the antennules, the presence aesthetascs, spinules by the method of Van de Velde[9]. Copepod specimens preserved in 80% ethyl alcohol and further confirmation for taxonomic identity were sent to the Department of Zoology, Acharya Nagarjuna University, Andhra Pradesh and the specimens were identified as *M. aspericornis*.

2.10. Culture of copepod in laboratory

Copepod was cultured, a system based on algae, protozoans such as paramecium, chilomonas, wheat seed and some lettuce particles are cultivated in laboratory in 30 litre fish tank. Protozoans serve as excellent food and provide support for adult copepod in dechlorinated water to culture more number of copepods for the experiment. *Paramecium* sp. prepared side by side from boiled rice straw water extract and commercial powdered fish foods used as food to the copepod. Copepod was cultured in dechlorinated water where temperature during the culture was kept at 28.8 °C with pH 7. Male and female copepod species from the colonies were separated using medicine dropper under a stereomicroscope. The copepods in container were covered with net cloth and gravid isofemale lines were pooled. The females continued to produce multiple batches of egg sacs. Each container should yield approximately 1 500-2 000 adult copepods.

2.11. Larval bioassays

Bioassays were performed in a 500 mL paper cup containing 250 mL (final volume) of water and one of five treatment solutions achieved by the addition of 2, 4, 6, 8, or 10 mL of leaf extract stock solution to the water in the cup. The resulting concentrations were 2%, 4%, 6%, 8% and 10% of plant extract, respectively. The controls contained an appropriate quantity of acetone in lieu of plant extract. For each assay, 50 larvae were placed in the (as yet) untreated water (along with a small quantity of food in the case of the larvae) and allowed to equilibrate for 1 h. Larval or pupal responses were observed 24 h after the addition of plant extract to the test unit. Each life stage was scored as dead when un-reactive to prodding with a small wooden dowel. A single bioassay series for each life stage included the five concentration of plant extract specfied above plus a control. The life stages tested were instar 1 (at 1-2 d after hatching) instars 2 through 4 (24-36 h after molting) and the pupa (24-36 h after pupation). Each series was replicated three times for each instar (n=72) and for pupae (n=18). Environmental conditions during larval and pupal bioassays were the same as for general mosquito rearing[10].

2.12. Predatory efficiency test

Each copepod species having capacity to killed *Ae. aegypti* larvae was assessed by placing single adult copepods in tissue culture plate wells (35 mm diameter, 18 mm deep) with newly hatched first instar larvae in the laboratory condition. The predatory nature and the rate of predatory efficiency of adult *M. aspericornis* on mosquito larvae were observed under a stereomicroscope. Hundred numbers of mosquito larvae (I to IV instars) and twenty numbers of adult copepod were introduced individually into the 500 mL glass beaker containing 250 mL of dechlorinated water and observed for whole day. The copepod attacked and killed *Ae. aegypti* larvae were observed under microscope. The numbers of dead larvae were counted at every 24 h at 26-28 °C. The glass beakers were checked without treatment (control) on first day, second day, third day, fourth day and fifth day and the number of prey consumed by the predator was checked and recorded. The mosquito larvae were replaced daily with the new ones. The experiment was held up with 4 trials and each trial consisted of four replicates. Predatory efficiency of a copepod was calculated by the following formula,

$$\text{Predatory efficiency} = \frac{\text{No. of prey/No. of predator introduced}}{\text{Total number of prey introduced}} \times 100$$

2.13. Predatory safety test

The predatory safety test was analysed to ascertain the survivability and safety of copepod after the treatment of *D. elata* extract. The effect of *D. elata* extract was tested against non-target predator, *M. aspericornis* and its nauplius was maintained in the laboratory condition at (27±3) °C and relative humidity. *M. aspericornis*, predator was released into 500 mL disposable bowl containing 250 mL dechlorinated water. The predators were exposed to different concentrations of plant extract from 2% to 10% in individual and combined treatment also. Five replicates were performed for each concentration along with untreated controls. The predator, *M. aspericornis* was observed for mortality, abnormalities, survival and swimming activity after 24 h treatment of plant. The exposed predator was

observed for a week, after the treatment of different concentration of *D. elata* extract in order to observe suitability of interaction for mosquito control. LC_{50} values were obtained by probit analysis and Suitability index (SI) or Predator safety factor (PSF) was calculated for the predator using the formula (Deo *et al*., 1988),

$$SI/PSF = \frac{LC_{50} \text{ of non-target organism}}{LC_{50} \text{ of target vector organism}}$$

Non-target organism= Copepod; Target organism =Mosquito

SI/PSF indicated that less value was lethal and higher value was susceptible.

Plant extract was very less harmful to the predator.

2.14. Statistical analysis

The data gets from the bioassays and predation tests were analyzed using the SPSS software package. The DMRT and *t*-test were used to test for significant differences in mortality. The analytical data together with Tables are presented in appropriate places in the thesis.

3. Results

Table 1 provides the ethanolic extract of *D. elata* on various stages (I, II, III, IV and pupae) of *Ae. aegypti*. Considerable mortality was evident after the treatment of *D. elata* for all larval stages. Mortality was increased as concentration increased. The percentage mortality of I, II instar was 100% at 10% concentration and it was further observed and remarkable mortality in III & IV instar. Similar trend has been noted for all the instars (III and I). The effect on larval mortality was concentration dependent (Table 1). The LC_{50} (LC_{90}) values were 4.91 (8.13), 5.16 (8.44), 5.95 (7.76), 6.87 (11.23); regression equation values were as 0.399 1(−1.196 3); 0.391 7(−2.02 5); 0.335 8(−1.99 9); 0.294 2(−2.0232) and the curve fitted in the table and *Chi*-square values were 2.278, 2.653, 0.226, 0.325 in I to IV instar larvae treated with ethanolic extract of *D. elata*. The DMRT values are significant at 5% level. The *Chi*-square values were significant at $P<0.05$ level.

Table 2 provides the predatory efficiency of copepod against various larval instars of *Ae. aegypti*. High rate of predatory efficiency of adult *M. aspericornis* was in this experiment. There was no effect observed in the copepod movement. I and II instars of larvae were much preferred to the copepod to feed, when compared with later instars III and IV. The percentage of predation was 39.0%, 30.2%, 25.6%, 2.8% in I to IV instar larvae, respectively. Table 3 provides the predatory efficiency of adult *M. aspericornis* with different concentration of *D. elata* ethanol extract

Table 1
Larvicidal effect of ethanolic extract of *D. elata* against dengue vector, *Ae. aegypti*.

Instars	No. of Mosquito 50 (Death / 24h) Concentrations (%)					% of Mortality	% Value of LC_{50} LC_{90}	95% Confidential limit		Regr. Coeff. X(Y)	*Chi* square value (x^2)
	2	4	6	8	10			LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})		
I	8^{a}	16^{a}	32^{a}	44^{a}	50^{a}	100 (30.0±0.24)	4.91 (8.13)	4.40 (7.45)	5.40 (9.08)	0.399 1 (−1.196 3)	2.278
II	7^{ab}	15^{ab}	30^{b}	42^{b}	50^{a}	100 (28.8±0.24)	5.16 (8.44)	4.66 (7.74)	5.65 (9.41)	0.391 7 (−2.025 0)	2.653
III	5^{c}	12^{c}	26^{c}	37^{c}	46^{b}	96 (25.2±0.23)	5.95 (7.76)	5.40 (8.93)	6.50 (10.97)	0.335 8 (−1.999 0)	0.226
IV	4^{cd}	10^{d}	20^{d}	30^{d}	42^{c}	92 (21.2±0.24)	6.87 (11.23)	6.27 (10.15)	7.53 (12.87)	0.294 2 (−2.023 2)	0.325

LCL – Lower Confidential Limit, UCL– Upper Confidential Limit, data were expressed as mean±SE within the column followed by the same letter(s) are not significantly different at 5% level by DMRT; *Chi*-square value significant at $P<0.05$ level.

Table 2
Predatory efficiency of adult copepod, *M. aspericornis* against different larval instars of dengue vector, *Ae. aegypti*.

Larval instar	No. of copepod	% of Predatory efficiency in days (hours) 5	10	15	20	24	Percentage of predation	Predatory efficiency of a single copepod in days	Total Predation
Control	20	0±0	0±0	0±0	0±0	0±0	0	0	0
I	20	46.0±0.4	44.0±0.3	39.0±0.2	34.0±0.2	32.0±0.4	39.0±0.16	1.95	195
II	20	39.0±0.2	36.0±0.4	28.0±0.2	26.0±0.3	22.0±0.4	30.2±0.16	1.51	151
III	20	31.0±0.3	29.0±0.4	26.0±0.3	22.0±0.4	20.0±0.3	25.6±0.25	1.28	128
IV	20	4.0±0.3	4.0±0.4	3.0±0.2	2.0±0.3	1.0±0.2	2.8±0.10	0.14	14

Table 3
Combined study of predatory efficiency of *M. aspericornis* and *D. elata* against *Ae. aegypti*.

Concentration (%)	No. of larval instar	No. of copepod	Percentage of predatory efficiency in days (h) 5	10	15	20	24	Percentage of predation±S.E	Predatory efficiency of a single copepod in days	Total predation
Control	100	20	0±0	0±0	0±0	0±0	0±0	0±0	0	0
1.0	100	20	92.0±0.5	89.0±0.4	83.0±0.3	80.0±0.4	75.0±0.2	83.8±0.39	4.19	419
2.0	100	20	94.0±0.3	86.0±0.4	80.0±0.2	73.0±0.2	69.0±0.4	80.4±0.34	4.02	402
3.0	100	20	89.0±0.2	81.0±0.5	77.0±0.2	72.0±0.4	60.0±0.4	75.8±0.06	3.79	379
4.0	100	20	71.0±0.2	59.0±0.4	51.0±0.5	44.0±0.4	41.0±0.3	53.2±0.09	2.66	266

against the third and fourth larval instar of *Ae. aegypti*. The predatory efficiency of adult *M. aspericornis* increased when the mosquito larvae were treated at various concentrations. No predatory effect was observed in untreated larvae. The predatory efficiency of copepod was not affected in the combined treatment of *D. elata*. The predatory efficacy of a single copepod on the *D. elata* at 1+20, 2+20, 3+20 and 4+20 treated larvae were the predatory efficiency at 4.19, 4.02, 3.79 and 2.66 larvae/day, respectively. The percentage of predation was 83.8%, 80.4%, 75.8% and 53.2%, respectively. As the concentration increasing the percentage of predation was neither increased nor decreased in the III and IV instar of mosquito. The copepod and plant extract were significantly reduced the mosquito larval population. The plant extract of *D. elata* was not affect the copepod predation and movement. The plant and copepod are joined together to rapidly kill the mosquito population and plant extract concentration does not affect the copepod survival.

4. Discussion

Crude extract of *Swartzia madagascariensis* fruits produced higher mortality in larvae of *Anopheles gambie* (Edwards) (*An. stephensi*) than larvae of *Ae. aegypti*, but was ineffective against larvae of *Culex quinquefasciatus* (*Cx. quinquefaciatus*) by Minijas and Sarda[11]. Sujatha examined the larvicidal activity of five plants, among which the extracts of *Acorus calamus* and *Bambusa arundanasia* were the most effective against *Cx. quinquefaciatus* and *An. stephensi*, respectively[12]. On the other hand, the extract of *Citrul medica* affected only larvae of *An. stephensi* and the extract of *M. longifolia* responses was ineffective against the species. Pandian have reported *Mentha piperita* to be highly effective in controlling the larvae of *Cx. quinquefasciatus* and the ethanolic extracts of *Solanum suratense*[13], *Azadirachta indica* and *Hydrocotyl javanica* exhibited larvicidal activity against *Cx. quinquefasciatus*[14-16].

In present study, the larvicidal activity was more pronounced with the ethanol extract of *D. elata* against *Ae. aegypti*. The potential lethal activity against *Ae. aegypti* was observed with *D. elata*, indicated that the presence of chemical composition such as glycosides, phenolic compounds, alkaloids and flavonoids in the species. Sivanarayan and Suryavathana showed that maximum activity was observed against all the species using *D. elata* and *P. cineraria* leaves, these plants can be useful, seems to be a potential source for arresting the growth and metabolic activities of various general bacteria and fungi, the presence of phytochemicals in study might be a factor for the antibacterial activity of *D. elata* and *Prosopis cineraria* leaves[17]. Differences in the larvicidal effects on *Ae. aegypti* among the stem distilled oils from the whole plants of *Tagets erecta* L., *Tagetes minuta* and *Tagete patula* L., have been reported and suggested that *Tagetes minuta* had the most potent larvicidal activity[18,19].

Babu and Murugan investigated that the larvicidal effect of resinous exudates from tender leaves of *Azadirachta indica*[20]. Vahitha have studied the larvicidal efficacy of *Pavonia zeylamica* and *Acacia feerruginea* against *Cx. quinquefasciatus*[21]. Effect of an insecticide and acetone leaf extracts of *Ania somnifera* (*A. somnifera*) and *Argemone mexicana* (*A. mexicana*) against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* was studied earlier. The insecticide effectively checked all mosquitoes population; both *A. somnifera* and *A. mexicana* were found to be more effective in controlling the mosquito's population. The early larval instars were highly susceptible to plant extracts than later stages. When the effects of two extracts were compared, it was discernible that the leaf extract of *A. somifera* was more effective in controlling the population of *Ae. aegypti* than the leaf extract of *A. mexicana*[22].

In the present study, the two solvent extract of *D. elata* showed higher larvicidal activity, but ethanol extract gives higher larvicidal and pupicidal activity than the acetone extract. Various parts of the *D. elata* plant (leaves flower and twig) were effective in controlling mosquito. The effects of various extracts were studied in a dose dependent manner. The methanol and ethanol flower extract of *Lantana camara* (*L. camara*) was found to have higher rate of larvicidal rate against *Ae. aegypti*, where as in the *Cx. quinquefasciatus* variety, the concentration of extracts have to be increased for better larvicidal effect and the leaf, flower extracts of *L. camara* obtained using different solvents were found to have larvicidal activity proposing the use of leaves as well as flowers of *L. camara* as a mosquito control agent[23]. Earlier, Venkatachalam and Murugan reported that *Toddalia asiatica* leaf extracts has larvicidal activity against all larvae and pupal stages of *Ae. aegypti* with LC_{50} and LC_{90} ranging from 47.90 to 61.28 and 93.98 to 116.22 mg/L, respectively[24].

The present study reported that the percentage of predation was highly reduced as the mosquito larvae grew older and most of the cyclopoids are predators, cyclopoids are the only copepods that prey on mosquito larvae. According to Lardeux *et al.*[25], *M. aspericornis* served as a good biocontrol agent against *Ae. aegypti* within a three-weeks time period whereas the maximum predatory capacity of *M. apericornis* was found to be 49.3 (mean value) and the minimum 39.3. *M. albidus* was a more efficient predator of younger than of older larvae. *M. aspericornis* consumed about 33 to 50 first instar larvae of *Ae. aegypti* within 24 h period. Copepods have a quick and well-developed snapping escape response to predators. It is much faster than that of either *Bosmina* or *Daphnia*, which sink passively as an escape response[26].

The current study showed predation upto 50% to 60 % on first, second and third instar. Plant inhibited the biological

system of larval instar of *Ae. aegypti* and thus the predatory efficacy was increased. Marten reported single-copepod predation rates of 90% on first instar mosquito larvae after 24 h[27]. A low predation rate of copepods on late instar mosquito larvae has been reported by previous authors[27-30]. Predation dropped considerably for 4 d and older larvae, which is consistent with previous observations for *Mesocyclops longisetus*[31]. Results demonstrate that *M. aspericornis* is an efficient predator of *Ae. aegypti* under laboratory conditions[32]. However, even at 4 d, there was close to 50% reduction in larval survival after 24 h with 5 copepods and more than 70% reduction with 10 copepods. These results also illustrate that *M. albidus* prefers to prey on younger larvae, but that it will increasingly attack older larvae as greater predator densities reduce the supply of younger ones.

Swimming behaviour, as well as size, has been shown to influence predation success in copepods[33,34]. These studies reported that copepod species preyed mainly on early instar larvae of mosquito and almost null predatory capacity upon older larvae was not surprising, considered that copepod cyclopoids are generally not large enough to kill third and fourth instar mosquito larvae. No predatory effect was happened predominantly on late instar of fourth due to the size and swimming behavior.

The present work, even in the normal container *M. aspericornis* preferred more on *Ae. aegypti* larva and kills upto 70%-80% of late instar larvae and there had no noticeable demand of food supply to the living copepod, which get nutrients from biodegradable plant and bacterial insecticide. Even predation with continuous supply of food shows better life cycle of *M. aspericornis*. But the size of the larva also shows disturbance to *M. aspericornis* for predation even at higher concentration and no predation was observed and less predatory effect in late instar of third compared to the early instars of first and second. Marten reported larger copepods, including many species of *Mesocyclops*, typically kill 95%-100% of the *Aedes* larvae in a container and cyclopoids reduced the numbers of third and fourth instars even more than they reduced the number of positive containers[27].

In the current study, nauplius and copepodite are available more food from plant and B.S and possibility of predation was noted against larval instar of *Ae. aegypti* and normal size containers are used. Predatory effect was observed better in nauplius and copepodite at various concentrations of plant and B.S. If the food supply in a container is poor, the best strategy is to add a small quantity of leaves or grain, and possibly the container with protozoa, to stimulate food production for the copepods. Copepods may fail to establish large numbers and eventually die out in containers (*e.g.*, flower vases, tires, or cement tanks) if the container is so clean that it provides little food[35,36]. In tropical waters, predatory cladocerans are absent but cyclopoid copepods are the dominant predators on small zooplankton[37]. Algae form part of the diet of many species, but cyclopoids fed on algae alone usually do not reproduce normally, and some species such as *Mesocyclops leuckarti* require a mixed diet including animal protein to form eggs[8,38-41].

In the present study somewhat correlated, more predatory efficiency of copepod within one or two days on 2nd and 3rd instars of larvae after the treatment of *D. elata* and *Bacillus sphaericus* whereas less predatory efficiency occured in 4th larval instar of *Ae. aegypti*. This activity might be due to enormous amount of food supply by the plant and survival in the plant extract and microbial contents. An emulsion of neem oil in water was found to be effective in controlling breeding of *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* in pools, tanks and coolers up to 2 to 3 weeks by Batra *et al*[42]. Murugan also reported that copepods are effective predators of first and second instars of mosquitoes but are not effective against the late instars[43]; hence the combined approach using botanicals which increases the predatory efficiency of copepods against the late instars and study also reveals treatment with neem, the copepods showed higher predation rate against *Ae. aegypti* larvae when compared to the predation without neem treatment.

Generally, the predator *M. aspericornis* consumed first and second instars in large numbers than third and fourth instars and due to the active movements, large size of the older larval instars that might be reduced to the predation rate of the copepods. In current study, little punctures and injuries to late instars of mosquitoes lead to constrained development and death. Mosquito of I, II and III instars were much preferred for by copepod. No predation observed in fourth later instars. The plant extract was non-toxic even at higher concentration to copepod, and the copepod observes any abnormalities such as sluggishness and reduced swimming activity after 24 h exposure. The exposed predators were also observed continuously for a week to understand the post treatment effect of this extract does not effect on survival and swimming behavior. To find any lethal effect on copepod and mosquito after combined treatment the suitability index was done for predator, copepod.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Plant biotechnology: Assessment and inventorisation of plant genetic resources for conservation and sustainable utilization in the region through biotechnological tools.

Microbial biotechnology: Microbial technology with special reference to improvement of abiotic-stress tolerance characteristics of agriculturally/ecologically important microbes.

Insect biotechnology: Involving insect-plant interactions, chemical and molecular ecology; development of eco-friendly insecticides and application of biotechnological tools for pest management.

Research frontiers

Molecular characterization of Bt isolates from Mizoram soils and their cry gene toxicity. Molecular Phylogeny of anopheline mosquito species of Mizoram and characterization of their resistant genes. Comparative phylogeny of few Nymphalid butterfly species distributed across North East India using mitochondrial and nuclear marker genes. Characterization of the cytotoxic protein pierisin from pierid butterflies. Biodiversity and molecular phylogeny of wild silk moths in Mizoram based on mitochondrial (16S rRNA and CoI) gene markers.

Related reports

The insecticidal activity and developmental study are most important for vector control filed. Most of the plant species are mosquitocidal activity and few are control developmental activity and inhibit the oval development. Hence this paper studied in this aspect and moreover, experimental plant species are further for phytochemical analysis.

Innovations & breakthroughs

Plant species of *A. alnifolia* and *V. negundo* inhibit of development of egg production in female mosquito. Most of the plant have insecticidal activity but this plant specifically contain phytochemical for arrest the developmental activity. Especially inhibit the moulting process, which means retain the juvenile hormone.

Applications

These plants species are commercially used for field study and application. The combination of this plants most effective and control mosquito aquatic community very effectively and is eco-friendly.

Peer review

This paper is significant for mosquito vector control especially insect not able to resistant. Synergistic study of plant species regulate the insect control mechanism is one of the significant study in this paper.

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